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㉑ Method and composition for making liposomes.

㉒ Mixtures of a hydrating agent such as arginine or glutamic acid and liposome-forming materials provide a pre-liposome gel which spontaneously forms highly stable liposomes in aqueous solution having very high capture efficiency.

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METHOD AND COMPOSITIONS FOR MAKING LIPOSOMES10 Background

This invention relates to novel liposome-forming compositions which provide a new method for forming liposomes. More specifically, this invention relates to the use of hydrating agents, compounds with at least two ionizable groups, with liposome-forming materials to make a gel which spontaneously forms liposomes when diluted with an aqueous solution.

General Description

20 Numerous processes and methods have been developed for making the different types and sizes of liposomes and for encapsulating active ingredient. Most of these methods have focused on the use of an organic solvent to ensure complete solubilization and uniform mixing of the 25 phospholipids and fatty acids prior to dispersion in an aqueous system. A second development was the use of ultrasonic irradiation to disperse the phospholipid/fatty acid material.

For example Robinson, Trans. Faraday Soc., 56:1260 30 (1960) and Papahadjopoulos, et al. [Biochim. Biophys. Acta, 135, 639 (1967)] describe formation of phospholipid dispersions from a two-phase ether/water system involving evaporation of the ether by bubbling nitrogen through the mixture. Similarly, chloroform has 35 been used by Chowhan, et al., Biochim. Biophys. Acta, 266:320 (1972) to insure a complete and thorough mixing of

1 the phospholipids prior to dispersion.

1 Ultrasonic dispersion, first described by D. Papahadjopoulos and N. Miller in Biochim. Biophys. Acta, 135:624 (1967) produces small unilamellar vesicles but the 5 technique is limited because of low encapsulation efficiency.

10 Batzri and Korn [Biochim. Biophys. Acta, 198:1015 (1973) have used the technique of injecting the lipids in an organic phase (ethanol) into an aqueous solution. Ether was used by Deamer and Bangham in essentially the 10 same technique [Biochim. Biophys. Acta, 443:619 (1976)].

15 Yet another technique involves a calcium-induced structural change in a lipid vesicle derived from or containing phosphatidylserine but is reported to have a relatively low encapsulation efficiency due to the method of reconstitution of the vesicle. See Papahadjopoulos, et al., Biochim. Biophys. Acta, 394:483 (1975) and H. Hauser, Trends in Pharm., Science 3, 274-77 (1982).

20 Several other patents set out methods for lipid vesicle formation of interest to this invention. U.S. patent No. 3,804,776 describes a method for producing oil and fat encapsulated amino acids for polypeptides by dispersing dry powders of the material in a molten mixture 25 of the fat or oil and then pouring the mixture into water. The encapsulated material is contained within relatively large droplets of lipid. Such vesicles are not suitable for IV injection and are limited to use only for oral administration.

30 Entrapment of certain drugs in lipid vesicles by freezing the aqueous phospholipid dispersion of the drug and lipid is described in U.S. patent No. 4,016,100.

35 Papahadjopoulos and Szoka, in U.S. patent No. 4,235,871, disclose a method for making liposomes where the vesicle-forming material is dissolved in an organic solvent and then mixed with an aqueous solution containing

1 the material to be encapsulated. An homogeneous water-in-oil emulsion is formed and the organic solvent is evaporated to give a gel-like mixture. This gel is then suspended in water to form the vesicles.

5 Another process of interest is disclosed in U.S. patent No. 3,932,657, which teaches the encapsulation of polyaminopolycarboxylic chelating agents, EDTA and TDPA. Yet another U.S. patent, 4,217,344, issued to Vanlerberghe, et al., notes that certain additives can be 10 combined with nonionic lipid compounds so as to modify the permeability and superficial charge of lipid vesicles. Among the several additives mentioned are polypeptides and proteins. Mackaness, et al. describe in U.S. Patent 4,192,859, contrast media containing liposomes as 15 carriers. The list of contrast agent salts includes arginine salt, cystein salt, glycine salt, glycyl glycine salt, and N-methylglucosamine salts. These materials are characterized as aliphatic and alicyclic amines which can be used to prepare water soluble salts of the various 20 contrast agents which may be employed in X-ray contrast agents.

An article in Science, March, 1984, by Janos Fendler makes reference to a number of synthetic surfactants which may be used in forming vesicles. Fendler references 25 quaternary ammonium and carboxylate, sulfate, sulfonate and phosphate zwitterionic materials which are referenced to the following literature articles: J. H. Fendler, Acc. Chem. Res., 13, 7 (1980); T. Kunitake and S. Shinkai, Adv. Phys. Org. Chem., 17, 435 (1980); T. Kunitake, et. al., J. Am. Chem. Soc., 103, 5401 (1981); J. H. Fuhrhop and J. 30 Matthieu, J. Chem. Soc. Chem. Commun., p. 141 (1983); and W. Salmon, et. al., Science, 221, 1047 (1983).

It has been discovered that liposomes are spontaneously formed when phospholipids and/or fatty 35 acids, liposome forming materials, are dispersed in an aqueous medium containing a hydrating agent. This class

1 of compounds, for the purpose of this invention, is  
1 illustrated by arginine and similar amino acids which have  
at least one ionizable functionality at both the alpha and  
omega termini of the molecule. These "hydrating"  
5 compounds will have either the same type of ionizable  
group on the molecule, both cationic, or both anionic, or  
have ionizable groups of opposite charge. It isn't  
required that the ionizable groups be on the alpha and  
omega carbons but such compounds represent the preferred  
10 embodiments of this invention.

10 In practical terms, liposomes formed using this  
invention are formulated as a "pre-liposome gel" referred  
to herein as a "gel" where a phospholipid and/or fatty  
acid mixture capable of forming liposomes is mixed with an  
15 appropriate, concentrated aqueous solution of the  
hydrating compound. This gel, upon dispersion in an  
aqueous medium efficiently and spontaneously forms  
liposomes without solvent evaporation, input of ultrasonic  
irradiation or any of the other means developed to insure  
20 proper formation of lipid vesicles, liposomes.

20 Liposomes made with hydrating agents are more stable  
than the ones produced by conventional methods, including  
those formed using organic solvents and ultrasonic  
energy. Liposome formulations having these hydrating  
agents suffer none of the solvent removal problems of the  
25 current technology nor are the liposomes beset by the  
non-uniform, destructive forces of ultrasonic irradiation  
inherent in the older methods.

30 Additionally, the pre-liposome gel can be dehydrated  
and stored for a substantial period of time and still be  
capable of spontaneously forming liposomes upon  
rehydration.

The pre-liposome gel is extraordinarily stable,  
stable enough to be autoclaved for sterilization.  
35 Furthermore, water-soluble or water-insoluble substances  
to be encapsulated can be added to the gel and will then

1 be incorporated into the liposomes upon dispersion of the  
5 gel. This capability has the effect of greatly enhancing  
the encapsulation efficiency.

Furthermore, it also has been discovered that the  
5 concentration of hydrating agent influences the size of  
the resulting liposomes in a predictable manner at a given  
pH. Correspondingly, varying the pH of the dispersing  
aqueous solution while holding the hydrating agent  
constant also influences the size of the liposomes  
10 produced in a predictable manner.

Thus, the present invention provides an easier, more  
convenient and predictable means for controlling vesicle  
size over methods previously available. This method also  
has no limitations on the concentrations of lipids in the  
15 preparation of liposomes.

Summary of the Invention

This invention relates to a liposome product made by  
dispersing in an aqueous medium, in a manner adequate to  
20 form liposomes, a composition comprised of:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is  
present in a molar ratio of between 1:20 and 1:0.05  
relative to the liposome-forming material;
- 25 c. water in an amount up to 300 moles relative to the  
solids; and
- d. optionally, a material to be encapsulated.

In a second aspect, this invention covers a  
composition capable of forming liposomes when dispersed in  
30 an aqueous medium, which composition comprises:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is  
present in a molar ratio of between 1:20 and 1:0.05  
relative to the liposome-forming material;
- 35 c. optional material to be encapsulated; and
- d. water in an amount up to 300 moles relative to the

1       solids.

5       In another aspect, this invention relates to a means for forming stable liposomes wherein the means comprises adding a hydrating agent to liposome-forming materials in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material and dispersing the mixture in an aqueous medium in a manner adequate to form liposomes. Alternatively, the hydrating agent, liposome-forming material and the substance to be encapsulated can be added 10 separately to the aqueous medium, then means for dispersion applied to form the liposomes.

#### SPECIFIC EMBODIMENTS

##### 15 Definitions

For the purpose of this invention, a hydrating agent means a compound having at least two ionizable groups, preferably of opposite charge, one of which is capable of forming an easily dissociative ionic salt, which salt can 20 complex with the ionic functionality of the liposome-forming material. The hydrating agent inherently does not form liposomes in and of itself. Such agent will also be physiologically acceptable, i.e., it will not have any untoward or deleterious physiological effect on the 25 host to which it is administered in the context of its use in this invention.

Complexing in this context denotes the formation of dissociative ionic salts where one functionality associates with the ionic functionality of the 30 liposome-forming material and the other functionality has hydrophilic properties which impart water-solubility to the resulting complex.

Hydrated complex means the complex formed between the hydrating agent and the liposome-forming material whereby 35 there is formed a specific semi-crystalline arrangement of molecules. Certain particular, specific spectral data

1 characterized the presence of this complex.

5 Mixtures of the hydrating agent and liposome-forming materials with certain, discrete amounts of water form a gel-like mass. When in this gel form, the hydrating agent and the liposome-forming material arrange into a "hydrated complex" which is a highly ordered liquid crystal. While the liquid crystal structure varies with pH and amount of hydrating agent, the liquid crystal structure remains. NMR spectroscopy confirms that the crystal structure 10 consists of multilamellar lipid bilayers and hydrophilic layers stacked together in alternating fashion. The  $^{31}\text{P}$ -NMR spectrum exhibits an anisotropic peak, further confirming the existence of multilamellar bilayers.

15 The word "liposome" has been proposed and accepted as the term to be used in the scientific literature to describe synthetic, oligolamellar lipid vesicles. Such vesicles are usually comprised of one or more natural or synthetic lipid bilayers surrounding an internal aqueous phase.

20 The phrase "liposome-forming material" refers to all natural and synthetic compounds which have one ionizable function and a hydrophobic component, a fatty component, such as the phospholipids, non-volatile fatty acids, non-volatile alkyl amines and the like which singly or in 25 combination form liposomes when dispersed in an aqueous medium. This definition is not intended to be limiting in its scope but is to be read to include all compounds capable of forming lipid vesicles, past, present and future.

30 Examples of liposome-forming materials include saponifiable and non-saponifiable lipids, e.g., the acyl glycerols, the phosphaglycerides, the sphingolipids, the glycolipids, etc. The fatty acids include saturated or unsaturated alkyl ( $\text{C}_8\text{--C}_{24}$ ) carboxylic acids, 35 mono-alkyl ( $\text{C}_8\text{--C}_{27}$ ) esters of  $\text{C}_4\text{--C}_{10}$  dicarboxylic acids (e.g., cholesterol hemi-succinic acid

1 and fatty acid derivatives of amino acids in which any  
N-acyl carboxylic acids also are included (e.g., N-oleoyl  
threonine, N-linoleoyl serine, etc.). Mono- or di-alkyl  
(C<sub>8</sub>~C<sub>24</sub>) sulfonate esters and mono- or di-alkyl  
5 (C<sub>8</sub>~C<sub>24</sub>) phosphate esters can be substituted for the  
fatty acids. Furthermore, mono- or di-acyl (C<sub>8</sub>~C<sub>24</sub>)  
glycerol derivatives of phosphoric acids and mono- or  
di-acyl (C<sub>8</sub>~C<sub>24</sub>) glycerol derivatives of sulfuric  
acids can be used in place of the fatty acids.

10 Additionally, the fatty acids also can be replaced by  
saturated or unsaturated alkyl amines (e.g., C<sub>8</sub>~C<sub>24</sub>  
NH<sub>2</sub>), C<sub>8</sub>~C<sub>24</sub> fatty acid derivatives of amines  
(e.g., C<sub>8</sub>~C<sub>24</sub> CONH~NH<sub>2</sub>), C<sub>8</sub>~C<sub>24</sub> fatty  
alcohol derivatives of amino acids (e.g., C<sub>8</sub>~C<sub>24</sub>  
15 OOC~NH<sub>2</sub>), and C<sub>8</sub>~C<sub>24</sub> fatty acid esters of amines  
(e.g., C<sub>8</sub>~C<sub>24</sub> COO~NH<sub>2</sub>).

Photopolymerizable lipids and/or fatty acids (or  
amines) (e.g., diacetylenic fatty acids) also can be  
included, which can provide a sealed liposome with  
20 cross-linked membrane bilayers upon photo-initiation of  
polymerization.

Although the primary components of these liposomes  
will be lipids, phospholipids, other fatty acids, there  
may also be added various other components to modify the  
25 liposomes' permeability. There may be added, for example,  
non-ionic lipid components such as polyoxy alcohol  
compounds, polyglyceral compounds or esters of polyoles,  
polyoxyalcolinolated alcohols; the esters of polyoles and  
synthetic lipolipids, such as cerebrosides. Other  
30 materials, such as long chain alcohols and diols, sterols,  
long chain amines and their quaternary ammonium  
derivatives; polyoxyethylenated fatty amines, esters of  
long chain amino alcohols and their salts and quaternary  
ammonium derivatives; phosphoric esters of fatty alcohols.  
35 polypeptides and proteins.

The composition of the liposome can be made of more

1 than one component of the various kinds of lipids, the fatty acids, alkyl amines, or the like, and the hydrating agents.

5 It also has been discovered that the lipid composition may not require the inclusion of the fatty acids (or the amines) or the hydrating agents to form the "pre-liposome gel" or liposomes, if the lipid component itself or the substances (e.g., medicaments, biologically active compounds, cosmetics, etc.) to be encapsulated 10 possess the aforementioned properties. For example, the mixture of dipalmitoylphosphatidylcholine (DPPC) and distearoyl phosphatidylethanolamine forms the "pre-liposome gel" or liposomes with aqueous glutamic acid solution and the mixture of DPPC and oleic acid with 15 aqueous epinephrine solution forms the "pre-liposome gel" and liposomes.

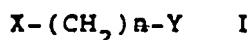
For pharmaceutical application as a liposome drug delivery system, however, the composition of phospholipids, oleic acid (or phosphatidylethanolamine) 20 and arginine or lysine (or glutamic acid and/or aspartic acid) was preferred.

When solids are referred to, the liposome-forming materials, hydrating agents, and material to be encapsulated if any, is what is being referred to.

25

#### Preferred Embodiments

The preferred hydrating agents of this invention are alpha amino acids having an ionizable omega substitution such as a carboxylate, amino, and guanidino function and 30 those compounds represented by the formula:



wherein

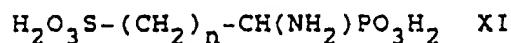
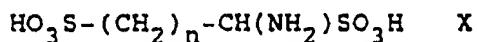
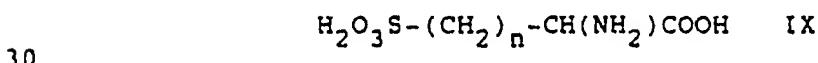
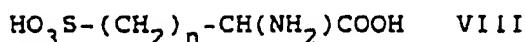
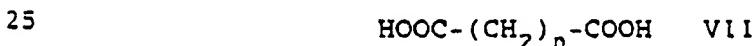
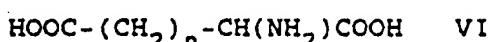
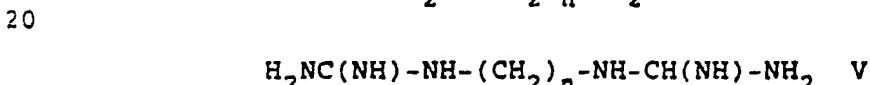
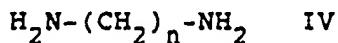
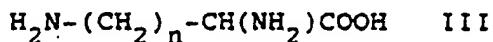
35 X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}_2\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  $\text{Z}\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;

1        Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  
CH(NH<sub>2</sub>)SO<sub>3</sub>Z or ZH(NH<sub>2</sub>)PO<sub>3</sub>Z<sub>2</sub> wherein Z is defined  
above; and

n is the integer 1-10; or

5        a pharmaceutically acceptable salt thereof. Also  
included in the list of preferred compounds are the  
N,N'-dialkyl substituted arginine compounds and similar  
compounds where the alkyl chain length is varied.

10      More preferred hydrating agents are the  
omega-substituted, alpha amino acids such as arginine, its  
N-acyl derivatives, homoarginine, gamma-aminobutyric acid,  
asparagine, lysine, ornithine, glutamic acid, aspartic  
acid or a compound represented by the following formulae:



35      wherein n is 2-4.

The most preferred compounds are arginine.

1 homoarginine, gamma-aminobutyric acid, lysine, ornithine, glutamic acid or aspartic acid.

About 1:20 molar ratio of hydrating agent relative to the liposome-forming material will provide the salutary 5 effects of this invention with an upper limit of about 1:0.05. The preferred concentration range for the hydrating agent is between a 1:2 to 1:0.5 molar ratio of the hydrating relative to the liposome-forming material.

The hydrating agents of this invention may be used 10 alone or as a mixture. No limitation is implied or intended in the use of mixtures of these hydrating materials.

As a practical matter, thus a matter of preference, if liposomes are prepared with liposome-forming materials 15 having a negative charge, it is preferred to use hydrating agents which contain at least one ionizable nitrogen, such as arginine, homoarginine, lysine, ornithine, and the like. Conversely, if the amphipatic materials used to form the liposomes are nitrogen-based, it is preferred to 20 use a di-acid such as glutamic acid, aspartic acid; any of the alkyl di-acids such as the simple di-acids such as valeric acid, caprylic, caproic, capric or the like; or those di-acids having two phosphate, or sulfate functionalities; or those di-acids having mixed 25 -COOH/-SO<sub>3</sub>H or -COOH/-PO<sub>3</sub>H<sub>2</sub> functions.

#### source of hydrating agents

The hydrating agents of this invention are listed in the catalogue of many chemical producers, can be custom 30 manufactured by such producers, or can be made by means known in the art.

Arginine, homoarginine, lysine, glutamic acid, aspartic acid, and other naturally occurring amino acids may be obtained by the hydrolysis of protein and 35 separation of the individual amino acids or from bacterial sources.

1        The compounds of formula II can be made by the method  
· of Eisele, K. et al, Justusliebigs. Ann. Chem., p 2033  
    (1975). Further information on several representative  
    examples of these compounds is available through their  
5        respective Chemical Abstracts Service numbers as follows:  
    norarginine, CAS # 14191-90-3; arginine, CAS # 74-79-3;  
    and homoarginine, CAS # 151-86-5.

10       'For representative examples of formula III, see for  
    2,4-diaminobutanoic acid CAS # 305-62-4 and for lysine  
10       CAS # 56-87-1.

15       Methods for making representative compounds of  
    formula IV are available from Chemical Abstracts as  
    follows: ethane diamine, CAS # 305-62-4; propane diamine -  
    54618-94-9; and 1,4-diaminobutane, CAS # 52165-57-8. See  
15       specifically Johnson, T.B., J. Am. Chem. Soc., 38, 1854  
    (1916).

20       Of the compounds of formula VI, glutamic acid is well  
    known in the art and is available from many commercial  
    sources. How to make other representative compounds is  
20       contained in the literature, for example:

25       2-aminohexadioic acid - CAS # 62787-49-9 and  
    2-aminoheptadioic acid - CAS # 32224-51-0.

25       Glutamic acid, the compound of formula VII where n is  
    2 is well known in the art and can be made by the method  
    of Maryel and Tuley, Org. Syn., 5, 69 (1925). Other  
    representative compounds in this group can be made  
    according to the art as referenced by the following CAS  
    numbers: hexadioic acid, CAS # 123-04-9 and heptadioic  
    acid, CAS # 111-16-0.

30       Homocysteic acid is known in the art referenced by  
    CAS # 56892-03-6. The compound 3-sulfovaline is described  
    in the literature referenced by CAS # 23405-34-2.

Pre-liposome Gel

35       Mixtures of liposome-forming materials and one or  
    more hydrating agents with up to 300 moles of water

1 relative to the total liposome-forming material gives a  
1 gel which forms liposomes directly therefrom upon addition  
1 of an aqueous medium. This gel is labeled a  
5 pre-liposome gel because i.) of its structural  
5 characteristics which are essentially those of liposomes  
and, ii.) the gel's facility for being converted into  
5 liposomes upon dilution with an aqueous medium. Aqueous  
medium in excess of about 300 moles cause the beginning  
of liposome formation.

10 The structure of this gel is a highly ordered liquid  
crystal which forms an optically clear solution. The X,  
Y, and Z dimensions of the liquid crystal vary with the  
concentrations of hydrating agent at the constant pH as  
well as with the pH of the solution. By varying the  
15 hydrating agent concentration at constant pH or changing  
the pH while maintaining percentage of hydrating agent,  
the size and number of lamellae structures of the lipid  
bilayers of the subsequent liposome vesicles can be  
controlled.

20 The gel structure itself can accommodate up to  
approximately 300 moles of water per mole of lipid or  
fatty acid without disturbing the stability of the gel  
structure. The structure of the gel as determined by  
proton NMR spectroscopy is comprised of multilamellar  
25 lipid bilayers and hydrophilic layers stacked together in  
an alternating fashion. The <sup>31</sup>P-NMR spectrum of the  
same gel exhibits an anisotropic peak further confirming  
that the gel consists of a multilamellar bilayer.

This gel can be autoclaved, a convenient means of  
30 sterilization. Furthermore, the gel shows no  
discoloration and remains clear at room temperature for at  
least one year after being autoclaved. The gel can  
further be sterilized by filtration through an appropriate  
sterilization filter.

35 Upon dispersion of the gel into an aqueous medium,  
liposomes are efficiently and spontaneously produced.

1        The pre-liposome gel, with or without the material to  
be encapsulated, also can be dehydrated (lyophilized) and  
the powder rehydrated to form liposomes spontaneously,  
even after a long period of storage. This capability  
5 makes the invention particularly useful for administering  
water-sensitive medicaments where long term pre-use  
storage is needed.

Either water insoluble or water soluble chemicals,  
drugs, cosmetics, food materials and the like, can be  
10 incorporated into liposomes prepared using this material  
and by this method. Accordingly, the gel may be used as a  
delivery system for the administration of medicaments via  
oral, parenteral, topical, intravenous, suppository routes  
or any other route of drug or chemical delivery.

15      The use of these liposomes is not limited to human or  
mammalian use but can be used in any industrial,  
agricultural, pharmaceutical, cosmetic or chemical  
application where lipid vesicle encapsulation and  
administration of compounds or materials is warranted or  
20 useful.

The versatility of the present invention is  
illustrated, but not limited, by the following examples.

Example #1

25      Preparation of Liposomes (Gel Phase)

Dipalmitoylphosphatidylcholine, 3.0 grams, was  
weighed into a 50 ml beaker. Oleic acid 1.2 grams was  
added and mixed together to form a uniform paste.

Arginine 0.72 grams in 30 ml of distilled deionized  
30 water was added to the  
dipalmitoylphosphatidylcholine-oleic acid paste and heated  
to 45°C. With mixing by hand, the mixture formed a  
clear stable gel. The gel was stored and liposomes later  
formed by diluting the gel with phosphate buffered saline.

1

Example #2Preparation of Liposomes

Dipalmitoylphosphatidylcholine, 120 mg, and 24 mg of oleic acid were added together and mixed thoroughly until 5 a white homogeneous paste was observed.

Then 20 mg of arginine was dissolved into 60 ml of phosphate buffered saline (ionic strength = 0.15, pH = 7.4). The arginine-saline solution was added to the paste and heated to 40°C for 1/2 hour, or until a slightly 10 turbid solution was observed.

Example #3Large Scale Gel and Liposome Preparation

i). Gel Manufacture: To 50 grams of egg phosphatide 15 powder type 20 (Ashai Chemicals) was added 20 grams of oleic acid N.F. Mixing gave a white paste which was cooled to 4°C and ground into a fine powder. This powder was added to an aqueous solution containing 20 grams of arginine and 500 grams of distilled deionized 20 water. The mixture was mixed with a spatula as the solution was heated to about 35°C to help hydrate phospholipids. A homogeneous, slightly yellow gel was formed. This gel can be stored at 4°C or can be frozen and later reconstituted.

ii). Manufacture of Liposomes: The gel prepared in the preceding paragraph was taken from cold storage and returned to room temperature. It was then mixed with 2 liters of phosphate buffered saline, pH 7.4. A white opaque liposome solution was formed.

30

Example #4Liposome Formation from the Gel

A homogeneous paste of 1.0 gram of dipalmitoylphosphatidylcholine (DPPC) and 400 mg of oleic 35 acid was formed. Then 300 mg of arginine was mixed in 10 ml of phosphate buffered saline, heated to 45°C and

1 added to the DPPC/oleic acid paste to form liposomes.

Example #5

Pre-Liposome Gel

5 One gram of dipalmitoylphosphatidylcholine (DPPL) was mixed with 400 mg of oleic acid to form a homogeneous paste. 300 mg of arginine was mixed with 2 ml of water at 45°C until dissolved. The arginine solution was mixed with the DPPC/oleic acid paste at about 45°C to give a 10 thick gel. Liposomes formed when this gel was diluted with phosphate buffered saline.

Example #6

Cholesterol Containing Liposomes

15 Cholesterol, 15 mg, was mixed with 100 mg dipalmitoylphosphatidylcholine (DPPC) to form a homogeneous powder. Then 23 mg of oleic acid was added to the powder and thoroughly mixed to form a homogeneous paste. To make liposomes, 30 mg of arginine was added to 20 10 ml of phosphate buffered saline, heated to 40°C and added to the DPPC/cholesterol/oleic acid paste. The combination was mixed at 40°C to obtain liposomes.

Example #7

Palmitic Acid-Containing Liposomes

25 Dipalmitoylphosphatidylcholine (DPPC) 250 mg was mixed with 25 mg of palmitic acid to form a uniform powder. Then 80 mg of oleic acid was mixed with this powder and heated to 45°C with constant stirring until a 30 uniform paste was formed. Arginine 100 mg was dissolved in 25 ml of distilled deionized water and heated to 45°C. This arginine solution was added to the paste at 45°C and mixed until a uniform homogeneous gel was formed. The gel was diluted ten fold with phosphate 35 buffered saline to form liposomes.

1

Example #8Isostearic Acid-Containing Liposomes

Dipalmitoylphosphatidylcholine 100 mg was mixed with 50 mg of isostearic acid to form a uniform homogeneous 5 paste. An arginine solution of 50 mg of arginine in 2.0 ml of distilled deionized water was made and added to the isostearic acid paste and heated to 45°C. The mixture was mixed until a clear gel was formed. Liposomes are formed upon dilution with phosphate-buffered saline.

10

Example #9Oleoyl Threonine Containing Liposomes

Dipalmitoylphosphatidylcholine 125 mg and 75 mg of oleoyl threonine were added together and heated to 40°C 15 to form a paste. Then 2 ml of distilled deionized water was added with constant mixing at 40°C. A clear gel was formed which can be diluted with phosphate buffer saline at pH 5 to form liposomes.

20

Example #10Myristyl Amine Containing Liposomes

Dipalmitoylphosphatidylcholine 192 mg was added to 72 mg of myristyl amine and heated with constant mixing until a uniform paste was formed. Glutamic acid 65 mg in 5 ml 25 of distilled deionized water was added to the paste and heated until a gel was formed. Phosphate buffered saline was added to the gel to form liposomes.

30

Example #11DLPC Containing Liposomes

Dilaurylphosphatidylcholine (DLPC) 50 mg was mixed with 20 mg oleic acid to form a homogeneous paste. Arginine 20 mg was added to 10 ml of phosphate buffered saline, added to the paste and hand mixed until a turbid 35 liposome solution formed.

1

Example # 12Phosphatidylethanolamine-Glutamic Acid Liposomes

L-glutamic acid 32 mg was dissolved in 2.0 ml of distilled deionized water and the pH adjusted to 5.2 with 5 1.0 N sodium hydroxide. This solution was heated to 60°C, and 100 mg of phosphatidylethanolamine added. The solution was kept at 60°C with constant mixing until a uniform viscous gel was observed.

10 The phosphatidylethanolamine-glutamic acid gel was diluted 1/10 by phosphate buffered saline. Vesicular like structures are observed under phase contrast light microscopy.

Example #13

15

Dipivalylepinephrine Liposomes

One gram of dipalmitoylphosphatidylcholine was mixed with 396 mg of oleic acid, until a homogeneous paste formed. Then 400 mg of arginine in 20 ml of distilled deionized water was added to form a pre-vesicle clear gel.

20 To make the liposomes, 242 mg of dipivalylepinephrine was dissolved in 10 ml of distilled deionized water. Then 5 grams of the pre-vesicle gel was mixed with 5 grams of the dipivalylepinephrine solution after which 50 ml of phosphate buffered saline was added forming a liposome 25 solution.

Example #14Flurbiprofen Liposomes

To make these liposomes, 980 mg of 30 dipalmitoylphosphatidylcholine, 370 mg of oleic acid, and 320 mg of flurbiprofen (free acid) were mixed together until a homogeneous paste was observed. Then 510 mg of arginine in 10 ml of purified water was added to the paste and heated to 41°C with constant mixing for 30 minutes.

35 A clear pre-vesicle gel formed of which 5 grams was introduced into 50 ml of phosphate buffered saline and

1 mixed with a stir bar until a bluish translucent solution was observed.

Example #15

5

Levobunolol Liposomes

Thirty mg of dipalmitoylphosphatidylcholine and 15 mg of cholesterol were weighed into a 4 ml vial. Ten mg of linoleic acid was added and mixed together to form a uniform paste. Two ml of a 1% aqueous levobunolol 10 solution containing 10 mg of arginine was added to the paste and mixed together. Then 10 ml of phosphate buffer solution was added and heated to 45°C to form liposomes.

Example #16

15

Pilocarpine Liposomes

To 120 mg of dipalmitoylphosphatidylcholine was added 40 mg of oleic acid to form a homogeneous paste. Forty mg of pilocarpine free base was added to 10 ml of distilled deionized water. This solution was added to the paste and 20 heated to 45°C to form a pre-liposome gel. The resulting gel was diluted with 20 ml of phosphate buffered saline to form liposomes.

Example #17

25

Epinephrine Liposomes

Dipalmitoylphosphatidylcholine 250 mg was mixed with 100 mg of oleic acid to form a homogeneous paste. 50 mg of epinephrine, free base, was dissolved in 5.0 ml of distilled deionized water, heated to 40°C and added to 30 the dipalmitoylphosphatidylcholine/oleic acid paste. This solution was mixed until a homogeneous viscous creamy gel was observed. This gel was diluted 1/5 with phosphate buffered saline (pH 7.22) to form liposomes.

-20-

1

EXAMPLE #18Effect of Arginine Concentration on Liposome Size

To 502 mg of dipalmitoylphosphatidylcholine (DPPC) was added 10 microliters of (2-palmitoyl-1-C<sup>14</sup>) (0.1 mCi/ml) dipalmitoylphosphatidylcholine. Chloroform was added to effect complete mixing of the radioactivity and then evaporated. Oleic acid (OA), 195 mg, was then mixed into the lipid to form a paste. Five ml of distilled water containing 119 mg of arginine was added and mixed at 10.45°C to form a clear gel.

One gram of the gel was weighed into four different vials and arginine was added as follows:

| Sample Composition |                                |             |
|--------------------|--------------------------------|-------------|
| 15                 | Sample ID                      | DPPC:OA:Arg |
|                    | Vial 1 + 1 ml water            | (1:1:1)     |
|                    | Vial 2 + 1 ml of 50 mg/ml Arg  |             |
|                    | in H <sub>2</sub> O            | (1:1:3)     |
| 20                 | Vial 3 + 1 ml of 84 mg/ml Arg  |             |
|                    | in H <sub>2</sub> O            | (1:1:5)     |
|                    | Vial 4 + 1 ml of 192 mg/ml Arg |             |
|                    | in H <sub>2</sub> O            | (1:1:10)    |

25

One-half gram of each solution was diluted in 50 ml of phosphate buffered saline of pH 7.8.

The estimated weight diameter was obtained from a Sephracyl S-1000 column chromatographic analysis employing <sup>14</sup>C-isotope labelled DPPC. The effects are given in the following Table.

1

Table IIEffects of Arginine Concentration on Vesicle Size

|    | <u>System</u>             | <u>pH</u> | <u>Estimated Weight Diameter (nm)</u> |
|----|---------------------------|-----------|---------------------------------------|
| 5  | DPPC:OA:Arg<br>(1 :1: 1)  | 7.8       | ~220                                  |
|    | DPPC:OA:Arg<br>(1 :1: 3)  | 7.8       | ~140                                  |
| 10 | DPPC:OA:Arg<br>(1 :1: 5)  | 7.8       | ~90                                   |
|    | DPPC:OA:Arg<br>(1 :1: 10) | 7.8       | ~20                                   |

EXAMPLE #19

15

pH Effect on Vesicle Size

Additionally, the vesicle size can be varied by varying the pH of the aqueous buffer solution.

To 100 mg of dipalmitoylphosphatidylcholine (DPPC) was added 25 microliters of (2-palmitoyl-1-C<sup>14</sup>) (0.1 20 mCi/ml) dipalmitoylphosphatidylcholine. Chloroform was added to effect complete mixing of the radioactivity and then evaporated. Oleic acid (OA), 40.1 mg, was then mixed into the lipid to form a paste. One ml of a solution containing 24 mg/ml arginine in water was added to the 25 lipid mixture and mixed at 45°C to form a clear gel.

Two 100 mg aliquots of this gel were diluted in 10 ml of phosphate buffer at pH 9.0 and 7.4 respectively.

Again, the estimated weight diameter (A) was obtained from the Sephracyl S-1000 column chromatographic analysis 30 employing <sup>14</sup>C-isotope labelled dipalmitoylphosphatidyl-choline. Results are given in the following Table.

Table III  
pH Effects on Vesicle Size

|    | <u>System</u>            | <u>Estimated Weight</u> | <u>pH</u> | <u>Diameter (nm)</u> |
|----|--------------------------|-------------------------|-----------|----------------------|
| 5  | DPPC:OA:Arg<br>(1 :1: 1) | 7.4                     |           | ~300                 |
|    | DPPC:OA:Arg<br>(1 :1: 1) | 7.8                     |           | ~220                 |
| 10 | DPPC:OA:Arg<br>(1: 1 :1) | 9.0                     |           | ~25.4                |

Thus, a desired size of the liposomal vesicles can be prepared by varying the arginine concentration or the pH of the aqueous buffer solution.

EXAMPLE #20

Sterile liposomes may be prepared from the heat  
sterilized pre-liposome gel. Alternatively, the liposome  
gel or the liposomes may be sterile filtered through an  
appropriate sterilizing filter.

Liposomes prepared from DPPC:OA:Arg (1:1:2) at pH 8.0 were heat sterilized and stored at room temperature for approximately one year without adding antimicrobial agents and anti-oxidants. No bacterial growth, discoloration and precipitation were observed. Negative stain electron microscopic examination of the one year old liposomes revealed that the liposomal vesicles are stable.

**EXAMPLE #21**

Encapsulated sucrose latency was measured using  $\text{C}^{14}$ -sucrose encapsulated with the DPPC:OA:Arg (1:1:1) liposome system in aqueous phosphate buffer solution at pH 7.8. The result was presented in Table IV.

1 Table IV

| <u>Days</u> | <u>% Sucrose Latency</u> | <u>% Latency</u> |
|-------------|--------------------------|------------------|
| 0           |                          | 100              |
| 5           | 1                        | 97.4             |
|             | 3                        | 93.4             |
|             | 7                        | 91.4             |

10 Thus, the present liposome system has an excellent latency  
10 for drug delivery.

EXAMPLE #22Efficiency of Encapsulation

15 A number of drugs were encapsulated with 10 mg/ml  
15 DPPC:oleic acid:Arg (1:1:1) liposomes to illustrate  
medicament encapsulation for use as a drug delivery  
system. The results are presented in Table V.

20 Table V

| <u>Drugs</u>          | <u>Entrapment of Drugs</u> | <u>% Entrapment</u> |
|-----------------------|----------------------------|---------------------|
| <u>pH</u>             |                            |                     |
| Flurbiprofen          | 7.8 PBS                    | 90%                 |
| Dipivalyl Epinephrine | 7.1 PBS                    | 80%                 |

25 Example #23Lyophilized Liposomes

Oleic acid, 30.0 gm, and 7.5 gm of cholesterol U.S.P.  
were confected. Then 75.0 gm of phosphatide type 20  
30 powder (Asahi Chemical Co.) was mixed with the oleic  
acid/cholesterol mixture until an homogeneous paste was  
formed.

Then 15.0 gm of arginine (free base) was dissolved in  
183 gm of distilled, deionized water. This arginine  
35 solution was mixed slowly with the lipid paste to form a  
homogeneous gel. The gel pH was adjusted to 7.4 using 5.0

1 N HCl.

A 10.0 gm aliquot of this pre-liposome gel was transferred to a 10 ml vial and lyophilized. The resulting powder formed liposomes when diluted with 5 ml 5 of phosphate buffered saline.

EXAMPLE #24

Acid Stable Liposome Preparations

An example of acid stable liposomes employing this 10 invention is illustrated by liposomes prepared with the following materials: distearoylphosphatidylcholine dipalmitoyl phosphatidylcholine: oleic acid, arginine, and cholesterol. These materials were combined in a molar ratio of 1:2:2:2:0.2 as follows: Cholesterol, 20 mg., was 15 mixed with 144 mg. of oleic acid and treated to 40°C. DSPC, 200 mg., and 400 mg. of DPPC was added and mixed at 40°C. The mixture was stirred until a uniform homogeneous paste was formed.

Arginine, 88 mg., was dissolved in 1.15 g. of 20 deionized distilled water. This arginine solution was added to the lipid paste and mixed at about 45°C until a homogeneous pre-liposome gel formed. The pH of the gel was adjusted to various pH levels with 0.1N HCl. The gel was diluted 10-fold with 0.9% NaCl, forming vesicles.

25

Liposome Stability at pH 4.4

|    | <u>Time/Days</u> | <u>Size/(NMx10<sup>3</sup>)</u> |
|----|------------------|---------------------------------|
| 30 | 0                | 1.024                           |
|    | 3                | 1.136                           |
|    | 7                | 1.127                           |

35

16501

1  CLAIMS (BE, CH, DE, FR, GB, IT, LI, LU, NL and SE)

1. A liposome composition made by dispersing in an aqueous medium in a manner adequate to form liposomes:

5       a. liposome-forming material;

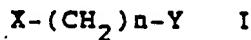
      b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and

      c. optionally material to be encapsulated.

10

2. The composition of claim 1 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, guanidino function or a pharmaceutically acceptable salt thereof or a compound of

15 the formula



wherein

X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  $\text{Z}\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;

20       Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$  or  $\text{ZH}(\text{NH}_2)\text{PO}_3\text{Z}_2$  wherein Z is defined above; and

n is the integer 1-10; or  
a pharmaceutically acceptable salt thereof.

25

3. The composition of claim 2 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

30       4. The composition of claim 3 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

35

1                    $\text{H}_2\text{NC}(\text{NH})-\text{NH}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{COOH}$    II.

$\text{H}_2\text{N}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{COOH}$    III.

5                    $\text{H}_2\text{N}-(\text{CH}_2)_n-\text{NH}_2$    IV.

$\text{H}_2\text{NC}(\text{NH})-\text{NH}-(\text{CH}_2)_n-\text{NH}-\text{CH}(\text{NH})-\text{NH}_2$    V.

$\text{HOOC}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{COOH}$    VI.

10                   $\text{HOOC}-(\text{CH}_2)_n-\text{COOH}$    VII,

$\text{HO}_3\text{S}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{COOH}$    VIII,

15                   $\text{H}_2\text{O}_3\text{S}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{COOH}$    IX,

$\text{HO}_3\text{S}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{SO}_3\text{H}$    X, or

$\text{H}_2\text{O}_3\text{S}-(\text{CH}_2)_n-\text{CH}(\text{NH}_2)\text{PO}_3\text{H}_2$    XI

20                  wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

5. The product of claim 4 wherein said hydrating  
25 agent is arginine, homoarginine, gamma-aminobutyric acid,  
lysine, or ornithine or a pharmaceutically acceptable salt  
thereof.

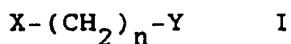
6. The composition of claim 4 wherein said hydrating  
30 agent is glutamic acid or aspartic acid or a  
pharmaceutically acceptable salt thereof.

7. A composition capable of forming liposomes when  
dispersed in an aqueous medium and the liposomes made  
35 therefrom, which composition comprises:

a. liposome-forming material;

1           b. a hydrating agent wherein the hydrating agent is  
present in a molar ratio of between 1:20 and 1:0.05  
relative to the liposome-forming material;  
5           c. optionally material to be encapsulated; and  
d. water in an amount up to 300 moles relative to  
the solids.

8. The composition of claim 7 wherein the  
hydrating agent is an alpha amino acid having an omega  
10 substitution which is a carboxylate, amino, or guanidino  
function or a pharmaceutically acceptable salt thereof,  
or a compound of the formula:

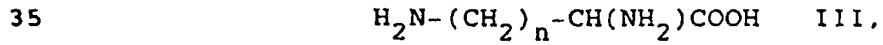
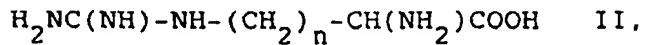


wherein

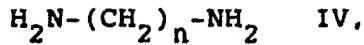
15           X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  
 $\text{Z}\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;  
Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  
 $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$  or  $\text{ZH}(\text{NH}_2)\text{PO}_3\text{Z}_2$  wherein Z is defined  
above; and  
20           n is the integer 1-10; or  
a pharmaceutically acceptable salt thereof.

9. The composition of claim 8 wherein the hydrating  
agent is present in an amount between 1:2 to 1:0.5 molar  
25 ratio relative to the liposome-forming material.

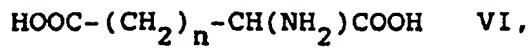
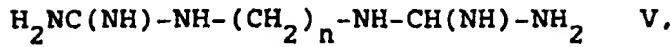
10. The product of claim 9 wherein said hydrating  
agent is arginine, homoarginine, or their N-acyl  
derivatives, gamma-aminobutyric acid, asparagine, lysine,  
30 ornithine, glutamic acid, aspartic acid or a compound of  
the formulae:



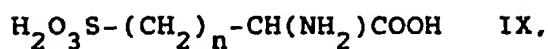
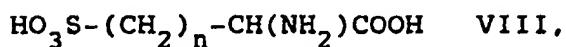
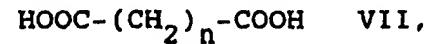
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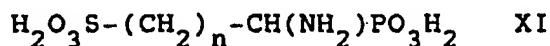
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10



15



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

20

11. The composition of claim 10 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

25

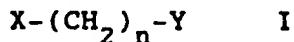
12. The composition of claim 10 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

30

13. A means for forming stable liposomes wherein the means comprises adding to liposome-forming materials before or at the time they are dispersed in an aqueous medium, a hydrating agent in a molar ratio of 1:20 to 1:0.05 relative to the liposome-forming material.

1        14. A substantially dry composition capable of  
      forming liposomes when dispersed in an aqueous medium  
      wherein the composition comprises:  
      (a) a liposome forming material;  
5        (b) a hydrating agent where the hydrating agent is  
          present in a molar ratio between 1:20 and 1:0.05  
          relative to the liposome forming material; and  
      (c) optionally material to be encapsulated,  
      which composition is prepared by mixing in an aqueous  
10      medium the materials of (a), (b) and (c), and dehydrating  
      said mixture to give said substantially dry composition.

15      15. The composition of claim 14 wherein the  
      hydrating agent is an alpha amino acid having an omega  
      substitution which is a carboxylate, amino, or guanidino  
      function or a pharmaceutically acceptable salt thereof,  
      or a compound of the formula:



wherein

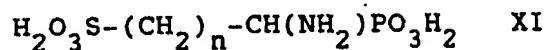
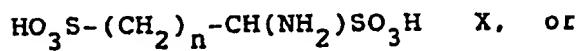
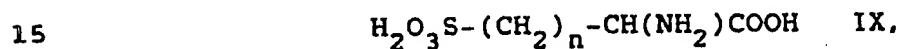
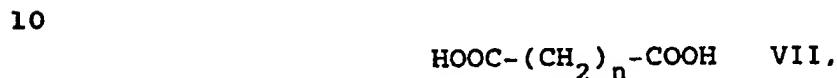
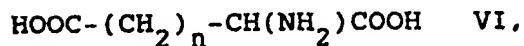
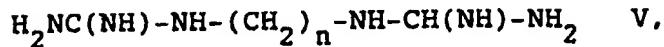
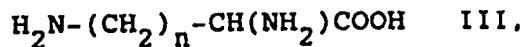
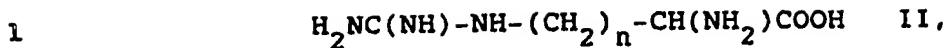
20      X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}_2\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  
       $\text{Z}\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;

      Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  
       $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$  or  $\text{Z}\text{H}(\text{NH}_2)\text{PO}_3\text{Z}_2$  wherein Z is defined  
      above; and

25      n is the integer 1-10; or  
      a pharmaceutically acceptable salt thereof.

30      16. The composition of claim 15 wherein the  
      hydrating agent is present in an amount between 1:2 to  
      1:0.5 molar ratio relative to the liposome-forming  
      material.

35      17. The product of claim 16 wherein said hydrating  
      agent is arginine, homoarginine, or their N-acyl  
      derivatives, gamma-aminobutyric acid, asparagine, lysine,  
      ornithine, glutamic acid, aspartic acid or a compound of  
      the formulae:



20                  wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

25                  18. The composition of claim 17 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

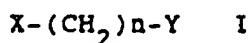
30                  19. The composition of claim 17 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

CLAIMS (AT)

1. A method for making a liposome composition which method comprises dispersing in an aqueous solution in a manner adequate to form liposomes:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and
- c. optionally material to be encapsulated.

2. The method of claim 1 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula



wherein

X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}_2\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  $\text{Z}_2\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;

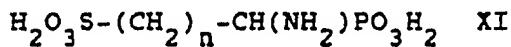
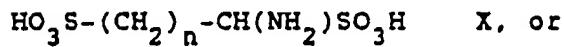
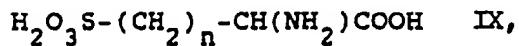
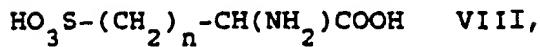
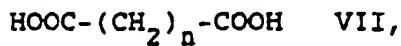
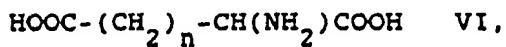
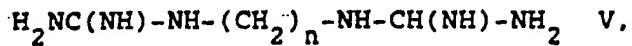
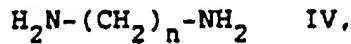
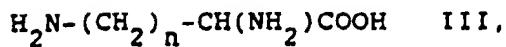
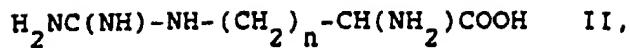
Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$  or  $\text{ZH}(\text{NH}_2)\text{PO}_3\text{Z}_2$  wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

3. The method of claim 2 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

4. The method of claim 3 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

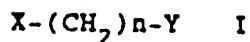
5. The method of claim 4 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

6. The method of claim 5 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

7. A method for making a liposome composition which method comprises dispersing in an aqueous solution in a manner adequate to form liposomes, a composition comprised of:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and
- c. water in an amount up to 300 moles relative to the solids; and
- d. optionally, material to be encapsulated.

8. The method of claim 7 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, or guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula



wherein

X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}_2\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  $\text{Z}\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;

Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$  or  $\text{ZH}(\text{NH}_2)\text{PO}_3\text{Z}_2$  wherein Z is defined above; and

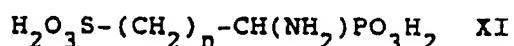
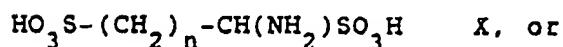
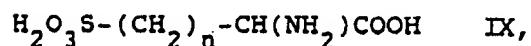
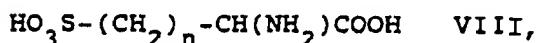
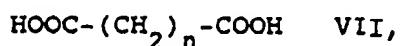
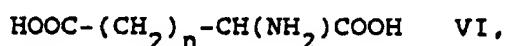
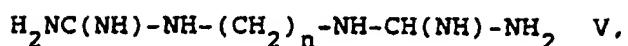
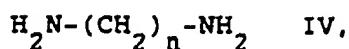
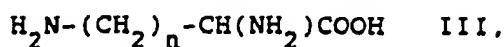
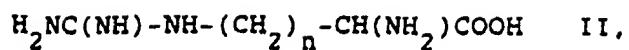
n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

9. The method of claim 8 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

10. The method of claim 9 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

-34-



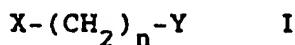
wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

11. The method of claim 10 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

12. The method of claim 10 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

1        13. A method of preparing a substantially dry  
composition capable of forming liposomes when dispersed  
in an aqueous medium wherein the composition comprises:  
5            (a) a liposome forming material;  
          (b) a hydrating agent where the hydrating agent is  
          present in a molar ratio between 1:20 and 1:0.05  
          relative to the liposome forming material; and  
          (c) optionally material to be encapsulated,  
which method comprises mixing in an aqueous medium the  
10    materials of (a), (b) and (c), and dehydrating said  
mixture to give said substantially dry composition.

14. The method of claim 13 wherein the hydrating  
agent is an alpha amino acid having an omega substitution  
15    which is a carboxylate, amino, or guanidino function or a  
pharmaceutically acceptable salt thereof, or a compound  
of the formula:

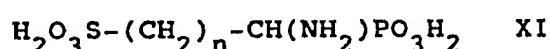
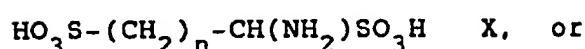
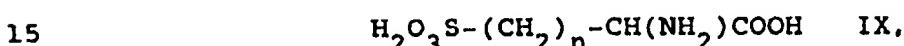
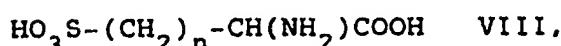
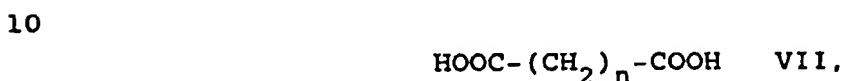
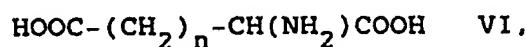
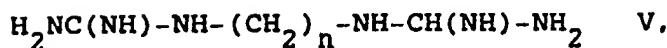
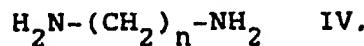
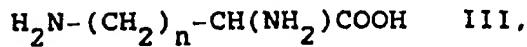


wherein

20    X is  $\text{H}_2\text{N}-\text{C}(\text{NH})-\text{NH}-$ ,  $\text{H}_2\text{N}-$ ,  $\text{Z}\text{O}_3\text{S}-$ ,  $\text{Z}_2\text{O}_3\text{P}-$ , or  
 $\text{Z}\text{O}_2\text{C}-$  wherein Z is H or an inorganic or organic cation;  
Y is  $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$ ,  $-\text{NH}_2$ ,  $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$ ,  $-\text{COOH}$ ,  
 $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$  or  $\text{Z}\text{H}(\text{NH}_2)\text{PO}_3\text{Z}_2$  wherein Z is defined  
above; and  
25    n is the integer 1-10; or  
a pharmaceutically acceptable salt thereof.

15. The method of claim 14 wherein the hydrating  
agent is present in an amount between 1:2 to 1:0.5 molar  
30    ratio relative to the liposome-forming material.

16. The method of claim 15 wherein said hydrating  
agent is arginine, homoarginine, or their N-acyl  
derivatives, gamma-aminobutyric acid, asparagine, lysine,  
35    ornithine, glutamic acid, aspartic acid or a compound of  
the formulae:



20                  wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

25                  17. The method of claim 16 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

30                  18. The method of claim 16 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.



| DOCUMENTS CONSIDERED TO BE RELEVANT  |  |                                  | EP 86306014.1   |
|--|--|----------------------------------|---|
| Category   | Citation of document with indication, where appropriate, of relevant passages  | Relevant to claim                | CLASSIFICATION OF THE APPLICATION (Int. Cl.4)   |
| A  | <p>DE - A1 - 2 706 705 (TAKEDA CHEMICAL INDUSTRIES)</p> <p>* Claims 1,17-19,26; page 11, lines 16-17; page 12, lines 29-31; examples 39,40 *</p> <p>--</p>   | 1-5,7-11,13-18                   | A 61 K 9/50<br>A 61 K 9/52<br>A 61 K 9/10<br>A 61 K 47/00<br>B 01 J 13/02<br>/A 61 K 31/195 |
| A  | <p>EP - A1 - 0 120 722 (PARFUMS CHRISTIAN DIOR)</p> <p>* Examples 7,8,9 *</p> <p>--</p>  | 1-4,7-10,13                      |   |
| X  | <p>EP - A1 - 0 087 993 (PARFUMS CHRISTIAN DIOR)</p> <p>* Claims 1,7,8; examples 11,12; page 17, lines 4-13 *</p> <p>--</p>   | 1-4,6-10,12,13                   |   |
| X  | <p>EP - A2 - 0 092 453 (THE LIPOSOME CORPORATION)</p> <p>* Abstract; claims 1,10,14,16, 19,28,29,31,32; page 11, line 23 - page 14, line 18; page 35, line 32 - page 36, line 12; sections 6.1-6.3 *</p> <p>--</p> | 1,7,13                           | TECHNICAL FIELDS<br>SEARCHED (Int. Cl 4)  |
| X  | <p>US - A - 3 957 971 (W.S.OLEUIACZ)</p> <p>* Abstract; claims 1,11,12; column 5, lines 18-45 *</p> <p>--</p>  | 1-4,6-13                         | A 61 K 9/00<br>B 01 J 13/00   |
| X  | <p>US - A - 3 932 657 (Y.E.RAHMANN)</p> <p>* Abstract; column 3, line 44 - column 4, line 33 *</p> <p>--</p>   | 1,7,13                           |   |
| The present search report has been drawn up for all claims   |  |                                  |   |
| Place of search  |  | Date of completion of the search | Examiner  |
| VIENNA   |  | 14-11-1986                       | MAZZUCCÒ  |
| CATEGORY OF CITED DOCUMENTS  |  |                                  |   |
| X : particularly relevant if taken alone<br>Y : particularly relevant if combined with another document of the same category<br>A : technological background<br>O : non-written disclosure<br>P : intermediate document  |  |                                  |   |
| T : theory or principle underlying the invention<br>E : earlier patent document, but published on, or after the filing date<br>D : document cited in the application<br>L : document cited for other reasons<br>& : member of the same patent family, corresponding document |  |                                  |   |



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EP 86306014.1

| DOCUMENTS CONSIDERED TO BE RELEVANT  |  |                                  | CLASSIFICATION OF THE APPLICATION (Int. Cl.4) |
|--|--|----------------------------------|---|
| Category   | Citation of document with indication, where appropriate, of relevant passages  | Relevant to claim                |   |
| X  | <u>WO - A1 - 84/02 076</u> (FLUID-CARBON INTERNATIONAL AB)<br>* Abstract; claims 1,2,4,19,26-28,32; examples 4,6 *<br>-- | 1,7,13                           |   |
| X  | <u>GB - A - 2 047 535</u> (A.NATTERMANN & CIE GMBH)<br>* Claims 1,3,5; examples 1,2 *<br>--                              | 1,7,13<br>14                     |   |
| X  | <u>GB - A - 2 041 871</u> (FARMITALIA CARLO ERBA S.P.A.)<br>* Abstract; claims 7,8; examples 1,2 *<br>--                 | 1,7,13<br>14                     |   |
| X  | <u>DE - A1 - 2 856 333</u> (A.NATTERMANN & CIE GMBH)<br>* Claims 1-4,7-9; examples 2,4 *<br>--                           | 1-5,7-<br>11,13-<br>18           | TECHNICAL FIELDS<br>SEARCHED (Int. Cl.4)      |
| A  | <u>AT - B - 356 278</u> (F.HOFFMANN - LA ROCHE & CO AKTIENGESELLSCHAFT)<br>* Claim 1 *<br>--                             | 1,2,7,<br>8,13                   |   |
| A  | <u>DE - A1 - 2 656 333</u> (K.THEURER)<br>* Example 2 *<br>--  | 1,2,4,<br>5,7,8,<br>10,11,<br>13 |   |
| A  | <u>CH - A - 498 627</u> (K.LARSSON)<br>* Claims 1,2; column 3, lines 22-31,60-67; column 4, lines 22-26 *<br>--          | 1,7,13                           |   |
| The present search report has been drawn up for all claims                       |  |                                  |   |
| Place of search  | Date of completion of the search   | Examiner                         |   |
| VIENNA   | 14-11-1986   | MAZZUCCO                         |   |
| CATEGORY OF CITED DOCUMENTS  |  |                                  |   |
| X : particularly relevant if taken alone   | T : theory or principle underlying the invention   |                                  |   |
| Y : particularly relevant if combined with another document of the same category | E : earlier patent document, but published on, or after the filing date  |                                  |   |
| A : technological background   | D : document cited in the application  |                                  |   |
| O : non-written disclosure   | L : document cited for other reasons   |                                  |   |
| P : intermediate document  | & : member of the same patent family, corresponding document   |                                  |   |

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EP 86306014.1

| DOCUMENTS CONSIDERED TO BE RELEVANT   |   |                                  | CLASSIFICATION OF THE APPLICATION (Int. Cl.4) |
|---|---|----------------------------------|---|
| Category  | Citation of document with indication, where appropriate, of relevant passages   | Relevant to claim                |   |
| A   | <p>US - A - 4 174 296 (G.S.KASS)</p> <p>* Claim 1 *</p> <p>---</p>  | 1                                |   |
| A   | <p>EP - A2 - 0 130 577 (DAIICHI SEIYOKU CO. LTD.)</p> <p>* Pages 4,5,8; abstract; claims 1,4-7,11-13 *</p> <p>---</p>                               | 1,7,13                           |   |
| A   | <p>EP - A2 - 0 102 324 (CIBA-GEIGY AG)</p> <p>* Abstract; claims 1,17; page 23, lines 18-23; page 24, line 22 - page 25, line 15 *</p> <p>-----</p> | 1,2,7,8,13,14                    |   |
| The present search report has been drawn up for all claims  |   |                                  | TECHNICAL FIELDS SEARCHED (Int. Cl.4)         |
|   |   |                                  |   |
|   |   |                                  |   |
|   |   |                                  |   |
|   |   |                                  |   |
|   |   |                                  |   |
| Place of search   |   | Date of completion of the search | Examiner                                      |
| VIENNA  |   | 14-11-1986                       | MAZZUCCO                                      |
| CATEGORY OF CITED DOCUMENTS   |   |                                  |   |
| <p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p>  |   |                                  |   |
| <p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p> |   |                                  |   |